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Conversion of 3-nitro-1-propanol (miserotoxin aglycone) to cytotoxic acrolein by alcohol dehydrogenase

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Experiments from this laboratory have demonstrated that appropriate nitro aliphatic compounds may serve as highly selective enzyme inactivators [1–3]. Our interest in 3-nitro-1-propanol stems from our experiments with the toxic antibiotic 3-nitropropionic acid, which irreversibly inactivates mitochondrial succinate dehydrogenase (EC 1.3.99.1) in a "suicide" reaction [2–4]. So-called loco weeds and poison vetches of the *Astragalus* genus are generally richer in 3-nitro-1-propanol than in 3-nitropropionate [5], and we have speculated that the toxicity of the alcohol may involve its metabolism to a carboxylic inhibitor of the Krebs cycle [1–3]. In some cases, however, 3-nitro-1-propanol is more toxic than 3-nitropropionate [5–7], and another mechanism occurred to us by which the nitro alcohol may be metabolized to an enzyme inactivator (Fig. 1). Upon enzymatic oxidation to the aldehyde level, the nitro compound is expected to spontaneously decompose into nitrite and acrolein, well-known mutagens and metabolic inhibitors with mechanisms of biological action which are understood in some chemical detail. Inorganic nitrite oxidizes hemoglobin [8] and, especially under acidic conditions, reacts with nucleic acids [9] and reacts with amines or amides to afford *N*-nitroso species which subsequently react with macromolecules [10]. Acrolein reacts directly with macromolecules [11, 12], is a crosslinking agent [13], and is one of the pharmacologically active metabolites of cyclophosphamide [14]. This communication reports that 3-nitro-1-propanol is a substrate for hepatic alcohol dehydrogenase (EC 1.1.1.1) and that the product does decompose into nitrite and acrolein under mild conditions.

Nitropropanol occurs naturally predominantly in the form of various glycosides such as miserotoxin (shown in Fig. 1), and it is known that the toxic action of miserotoxin requires the facile liberation of the toxic alcohol by enteral glycosidases [15]. Thus, the action of a glycosidase on miserotoxin followed by the action of a dehydrogenase may constitute an example of "lethal synthesis" [16]. Other aldehydes known to generate acrolein under mild conditions include those derived through enzymatic oxidation of cyclophosphamide [14] or of spermine [13].

3-Nitro-1-propanol was synthesized [17] from 3-bromo-1-propanol (Aldrich Chemical Co., Milwaukee, WI) and identified by its chromogenic reaction with *p*-nitrophenyldiazonium chloride [18] and by the electronic spectrum of its nitronate (E_{231} = 9300 in 10 mM aqueous KOH). Horse liver alcohol dehydrogenase, NAD, and glutathione were obtained from the Sigma Chemical Co., St. Louis, MO.

The alcohol dehydrogenase reaction was monitored with either a Cary 15 or a Gilford recording spectrophotometer by means of the absorbance of NADH (E_{340} = 6220). One unit of dehydrogenase was that which generated 1.0 μmole acetaldehyde/min in a solution containing 20 mM ethanol, 0.50 mM NAD, 1.0 mM EDTA, and 100 mM potassium phosphate at pH 7.4 and 25°. Inorganic nitrite was determined by means of the chromogenic reaction of *N*-(1-naphthyl)ethylenediamine with sulfanilamide diazotized by the nitrite [19]. Enzymatic reduction of benzaldehyde coupled to the oxidation of nitropropanol was followed by means of the ultraviolet absorbance (E_{242} = 13,800) of benzaldehyde extracted into cyclohexane from acid-quenched aliquots of the reaction mixture [20]. Thiol groups were colorimetrically titrated by means of Ellman's reagent (ΔE_{412} = 13,600) [21].

Steady-state kinetic parameters were measured in solutions containing an alcohol substrate, 0.50 mM NAD, alcohol dehydrogenase (0.008 to 0.041 units/ml), 1.0 mM EDTA, and 100 mM potassium phosphate at pH 7.4 and 25°. The nitro compound is a weak carbon acid (pK'_a =8.6) that is slow to dissociate at this pH [22] and was present at its equilibrium state of ionization. The maximum initial velocity seen with 3-nitro-1-propanol as substrate was 21 per cent of that obtained with ethanol. Half-maximum rates of oxidation were seen with 66 μM nitropropanol and with 890 μM ethanol. Increasing the nitropropanol concentration above 300 μM decreased the reaction velocity, but marked substrate inhibition of this enzyme is also known to occur with 1-propanol and 1-butanol [23]. In contrast to yeast alcohol dehydrogenase and other enzymes, the alcohol dehydrogenase from horse liver is remarkably resistant to inactivation by acrolein [11, 12, 20].

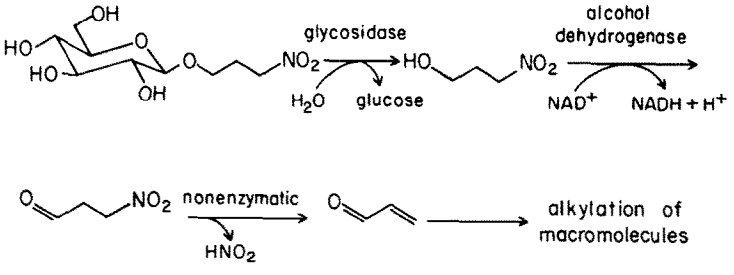

Fig. 1. Lethal synthesis of acrolein from miserotoxin, the β-D-glucoside of 3-nitro-1-propanol.

To estimate the rate of decomposition of the aldehyde derived from 3-nitro-1-propanol, we rapidly oxidized a sample of the alcohol by means of a large quantity of alcohol dehydrogenase in the presence of a catalytic quantity of NAD and an excess of the substrate benzaldehyde (Fig. 2). Aliquots of the reaction mixture were then assayed for inorganic nitrite. Simultaneously, production of acrolein was detected by means of its ability to capture the nucleophilic thiol group of glutathione. Upon modification, the glutathione no longer reduced Ellman's chromogenic reagent. As shown in Fig. 2, the end-points for nitrite production and glutathione comsumption corresponded to the quantity of 3-nitro-1-propanol oxidized. As expected, however, the nitrite production and thiol consumption proceeded more slowly than the prior enzymatic oxidation of nitropropanol.

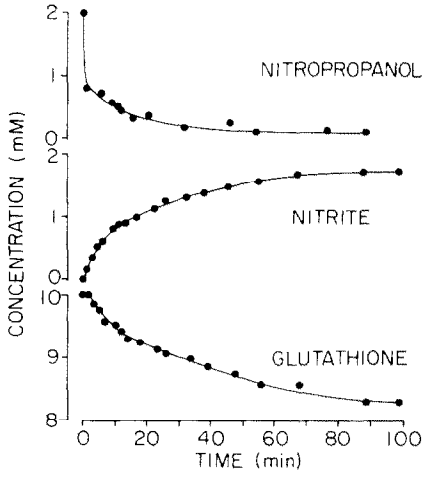

Fig. 2. Time courses of 3-nitro-1-propanol oxidation, inorganic nitrite production, and glutathione modification. The reaction mixture initially contained 2.0 mM nonionized 3-nitro-1-propanol, 0.10 mM NAD, 10 mM benzaldehyde, 6.4 units alcohol dehydrogenase/ml, 10 mM reduced glutathione, 1.0 mM EDTA, and 100 mM potassium phosphate at pH 7.4 and 25°. No nitrite production or thiol consumption occurred without the initial presence of the enzyme. The half-time for the ionization of 3-nitro-1-propanol to its equilibrium state in this buffer was 3.0 min.

The lethal synthesis of acrolein from miserotoxin resembles a scheme recently proposed to account for the organospecific carcinogenicity of cycasin (methylazoxymethanol β-D-glucoside) [24]. In that case, after liberation of methylazoxymethanol by a glycosidase, enzymatic dehydrogenation of that alcohol accelerated the rate of its decomposition into reactive methyldiazonium ions. By comparison, after liberation of 3-nitro-1-propanol from its glycosides, the action of alcohol dehydrogenase yielded labile 3-nitropropionaldehyde which spontaneously decomposed into inorganic nitrite and reactive acrolein. As indicated by both nitrite evolution and covalent modification of thiols (Fig. 2), the half-life of 3-nitropropionaldehyde was less than 20 min in 100 mM phosphate buffer at pH 7.4 and 25°.

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Department of Biochemistry and Biophysics
School of Medicine
University of Pennsylvania
Philadelphia, PA 19104, U.S.A.

THEODORE A. ALSTON
STEVEN P. SEITZ
HAROLD J. BRIGHT*

* Author to whom all correspondence should be addressed.

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